

RECEIVED
CENTRAL FAX CENTER

JUL 18 2007

Listing of Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

Claim 1 (previously presented): A competitive method for estimating the concentration in a sample of a *Bacillus anthracis* comprising the steps:

- a. intermixing a said sample suspected of containing said *Bacillus anthracis* polypeptide with a specific antibody to said polypeptide and a competitive reagent consisting of a *Bacillus anthracis* polypeptide labeled with a fluorochrome capable of binding to said specific antibody to produce a mixture;
- b. incubating said mixture for 15 seconds to 5 minutes;
- c. detecting binding interaction of said *Bacillus anthracis* polypeptide and antibody by fluorescence polarization, fluorescence lifetime or fluorescence resonance energy transfer.

Claim 2 (original): The method of claim 1, wherein said detection is by change in fluorescence polarization.

Claim 3 (original): The method of claim 1, wherein said detection is by change in fluorescence life-time.

Claim 4 (original): The method of claim 1, wherein said detection is by sensitized fluorescence of the acceptor or by quenching of donor fluorescence or by fluorescence depolarization.

Claim 5 (previously amended): The method of claim 2, wherein said method comprises the additional steps of:

- d. measuring the fluorescence polarization of a negative control solution of said fluorochrome-labeled competitive reagent, a positive control solution of said fluorochrome-labeled competitive reagent exposed to a known amount of said protein, or both, and
- e. comparing the measured fluorescence polarization of said mixture or said antibody detection mixture with the measured fluorescence polarization of said negative control solution, said positive control solution, or both.

Claim 6 (original): The method of claim 1, wherein said competitive reagent is native or recombinant *Bacillus anthracis* protein or fragments of said protein.

Claim 7 (original): The method of claim 1, wherein said protein can be detected down to less than 5 µg/ml or 6 nM within 5 minutes.

Claim 8 (original): The method of claim 1 wherein said sample is selected from the group consisting of broth culture media of growing *Bacillus anthracis* or bodily fluids.

Claim 9 (original): The method of claim 1, wherein said incubation step (b) occurs in less than 30 seconds for concentrated samples.

Claim 10 (original): The method of claim 1 wherein said incubation step (b) occurs in 4 to 5 minutes for low concentration samples.

Claim 11 (original): The method of claim 1 wherein said fluorochrome is pH independent.

Claim 12 (original): The method of claim 1 wherein said fluorochrome is selected from the group consisting of 7-AAD, Acridine Orange, Alexa 488, Alexa 532, Alexa 546, Alexa 568, Alexa 594, Aminonaphthalene, Benzoxadiazole, BODIPY 493/504, BODIPY 505/515, BODIPY 576/589, BODIPY FL, BODIPY TMR, BODIPY TR, Carboxytetramethylrhodamine, Cascade Blue, a Coumarin, Cy2, CY3, CY5, CY9, Dansyl Chloride, DAPI, Eosin, Erythrosin, Ethidium Homodimer II, Ethidium Bromide, Fluorescamine, Fluorescein, FTC, GFP (yellow shifted mutants T203Y, T203F, S65G/S72A), Hoechst 33242, Hoechst 33258, IAEDANS, an Indopyras Dye, a Lanthanide Chelate, a Lanthanide Cryptate, Lissamine Rhodamine, Lucifer Yellow, Maleimide, MANT, MQAE, NBD, Oregon Green 488, Oregon Green 514, Oregon Green 500, Phycoerythrin, a Porphyrin, Propidium Iodide, Pyrene, Pyrene Butyrate, Pyrene Maleimide, Pyridyloxazole, Rhodamine 123, Rhodamine 6G, Rhodamine Green, SPQ, Texas Red, TMRM, TOTO-1, TRITC, YOYO-1, vitamin B12, flavin-adenine dinucleotide, and nicotinamide-adenine dinucleotide.

Claim 13 (original): The method of claim 1 wherein said fluorochrome concentration is 1 nM or less and the sample millipolarization is increased or decreased by at least 10 mP.

Claim 14 (original): The method of claim 1 wherein the said antibody is polyclonal or monoclonal.

Claim 15 (original): The method of claim 8, wherein said bodily fluids are selected from the group consisting of saliva, oral rinse expectorant, oral fluid including oral mucosal transudate and gingival crevicular fluid, urine, sweat, tears, blood, serum, stool, gastric fluid, synovial fluid, phlegm, and other clinical and laboratory specimens and samples.

Claim 16 (withdrawn): A competitive method for estimating the concentration of specific antibody in a sample of bodily fluids to a protein from *Bacillus anthracis* selected from the group consisting

of protective antigen or PA, lethal factor or LF and edema factor or EF said competitive method selected from the group consisting of fluorescence polarization or FP, fluorescence lifetime or FLT and fluorescence resonance energy transfer or FRET the said competitive method of claim comprising the steps:

- a. intermixing a said sample suspected of containing said antibody with a competitive reagent labeled with a fluorochrome capable of binding to said specific antibody to produce an antibody detection mixture;
- b. incubating said antibody detection mixture for 15 seconds to 5 minutes;
- c. detecting binding interaction between said protein and antibody.

Claim 17 (withdrawn): The method of claim 16, wherein said detection is by change in fluorescence polarization.

Claim 18 (withdrawn): The method of claim 16, wherein said detection is by change in fluorescence life-time.

Claim 19 (withdrawn): The method of claim 16, wherein said detection is by sensitized fluorescence of the acceptor or by quenching of donor fluorescence or by fluorescence depolarization.

Claim 20 (withdrawn): The method of claim 16, wherein said method comprises the additional steps of:

- d. measuring the fluorescence polarization of a negative control solution of said fluorochrome-labeled competitive reagent, a positive control solution of said fluorochrome-labeled competitive reagent exposed to a known amount of said antibody, or both, and

e. comparing the measured fluorescence polarization of said mixture or said antibody detection mixture with the measured fluorescence polarization of said negative control solution, said positive control solution, or both.

Claim 21 (withdrawn): The method of claim 16, wherein said incubation step (b) occurs in less than 30 seconds for concentrated samples.

Claim 22 (withdrawn): The method of claim 16 wherein said incubation step (b) occurs in 4 to 5 minutes for low concentration samples.

Claim 23 (withdrawn): The method of claim 16 wherein said fluorochrome is pH independent.

Claim 24 (withdrawn): The method of claim 16 wherein said fluorochrome is selected from the group consisting of 7-AAD, Acridine Orange, Alexa 488, Alexa 532, Alexa 546, Alexa 568, Alexa 594, Aminonaphthalene, Benzoxadiazole, BODIPY 493/504, BODIPY 505/515, BODIPY 576/589, BODIPY FL, BODIPY TMR, BODIPY TR, Carboxytetramethylrhodamine, Cascade Blue, a Coumarin, Cy2, CY3, CY5, CY9, Dansyl Chloride, DAPI, Eosin, Erythrosin, Ethidium Homodimer II, Ethidium Bromide, Fluorescamine, Fluorescein, FTC, GFP (yellow shifted mutants T203Y, T203F, S65G/S72A), Hoechst 33242, Hoechst 33258, IAEDANS, an Indopyras Dye, a Lanthanide Chelate, a Lanthanide Cryptate, Lissamine Rhodamine, Lucifer Yellow, Maleimide, MANT, MQAE, NBD, Oregon Green 488, Oregon Green 514, Oregon Green 500, Phycoerythrin, a Porphyrin, Propidium Iodide, Pyrene, Pyrene Butyrate, Pyrene Maleimide, Pyridyloxazole, Rhodamine 123, Rhodamine 6G, Rhodamine Green, SPQ, Texas Red, TMRM, TOTO-1, TRITC, YOYO-1, vitamin B12, flavin-adenine dinucleotide, and nicotinamide-adenine dinucleotide.

Claim 25 (withdrawn): The method of claim 16 wherein said fluorochrome concentration is 1 nM or less and the sample millipolarization is increased or decreased by at least 10 mP.

Claim 26 (withdrawn): The method of claim 16, wherein specificity of detection is 96 – 99%.

Claim 27 (withdrawn): The method of claim 16, wherein said bodily fluids are selected from the group consisting of saliva, oral rinse expectorant, oral fluid including oral mucosal transudate and gingival crevicular fluid, urine, sweat, tears, blood, serum, stool, gastric fluid, synovial fluid, phlegm, and other clinical and laboratory specimens and samples.

Claim 28 (previously presented): The method of claim 1, wherein said *Bacillus anthracis* protein is selected from the group consisting of protective antigen, lethal factor and edema factor.